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(54) Title: METHODS FOR IN VIVO REDUCTION OF IRON LEVELS AND COMPOSITIONS USEFUL THEREFOR

(57) Abstract

In accordance with the present invention, there are provided methods for the *in vivo* reduction of free iron ion levels in a mammalian subject. The present invention employs a scavenging approach whereby free iron ions are bound *in vivo* to a suitable physiologically compatible scavenger. The resulting complex renders the free iron ions harmless, and is eventually excreted in the urine of the host. Further in accordance with the present invention, there are provided compositions and formulations useful for carrying out the above-described methods. An exemplary scavenger contemplated for use in the practice of the present invention is a dithiocarbamate-containing composition. This material binds to free iron ions, forming a stable, water-soluble dithiocarbamate-iron complex. Thus, the present invention relates to methods for reducing *in vivo* levels of free iron ions as a means of treating subjects afflicted with iron overload and non-iron overload diseases and/or conditions, such as thalassemia, anemia, hereditary hemochromatosis, hemodialysis, stroke and rheumatoid arthritis. Dithiocarbamate-containing scavengers are administered to a host in need of such treatment; these scavengers interact *in vivo* with free iron ions, thereby forming a stable dithiocarbamate-metal complex, which is then filtered through the kidneys, concentrated in the urine, and eventually excreted by the subject, thus reducing *in vivo* levels of free iron ions.

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Methods for In Vivo Reduction of Iron Levels and Compositions Useful Therefor

FIELD OF THE INVENTION

The present invention relates to methods for reducing iron levels in mammals. In a particular aspect, the present invention relates to methods for reducing free iron ion levels in mammals by administration of dithiocarbamates as scavengers of free iron ions in hosts undergoing anthracycline chemotherapy, as well as hosts suffering from iron overload or non-iron overload diseases and/or conditions, such as thalassemia, anemia, hereditary hemochromatosis, hemodialysis, stroke and rheumatoid arthritis. In a further aspect, the present invention relates to compositions and formulations useful in the methods disclosed herein.

BACKGROUND OF THE INVENTION

Iron is crucial for maintaining normal structure and function of virtually all mammalian cells (see, for example, Voest et al., in Ann. Intern. Med. 120:490-499 (1994) and Kontoghiorghes, G. J., in Toxicol. Letters 80:1-18 (1995)). Adult humans contain 3-5 g of iron, mainly in the form of hemoglobin (58%), ferritin/hemosiderin (30%), myoglobin (9%) and other heme or nonheme enzyme proteins (Harrison and Hoare, in Metals in Biochemistry, Chapman and Hall, New York, 1980).

Total iron levels in the body are regulated mainly through absorption from the intestine and the erythropoietic activity of the bone marrow. Upon absorption, iron is transported to various tissues and organs by the serum protein transferrin. Once transported to the target tissue or organ, iron is transported and stored intracellularly in the form of ferritin/hemosiderin. Under normal conditions, transferrin is about 30% saturated

with iron in healthy individuals, and an equilibrium is maintained between the sites of iron absorption, storage and utilization. The presence of these homeostatic controls ensures the maintenance of physiological levels of not only iron, but also other essential metal ions such as copper, zinc and cobalt.

Breakdown of these controls could result in metal imbalance and metal overload, causing iron overloading toxicity and possibly death in many groups of patients, especially those with idiopathic hemochromatosis (see, for example, Guyader et al., in Gastroenterol. 97:737-743 (1989)). Among its toxic effects, iron is known to mediate a repertoire of oxygen related free radical reactions (see, for example, Halliwell and Gutteridge, in Halliwell and Gutteridge, Free Radicals in Biology and Medicine, 2nd edition. Oxford: Clarendon Press, 15-19 (1989)). For example, iron, particularly in the form of free iron ions, can promote the generation of reactive oxygen species through the iron-catalyzed Haber-Weiss reaction (see, for example, Haber and Weiss, in Proc. R. Soc. Ser. A. 147:332 (1934)) as follows:

$$Fe^{3+} + \cdot O_2^- \longrightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + \cdot OH + OH^-$$

The net result of these reactions is as follows:

$$\cdot O_2^- + H_2O_2 \longrightarrow \cdot OH + OH^- + O_2.$$

The Haber-Weiss reaction is seen to produce the hydroxyl radical ($\cdot OH$), a highly potent oxidant which is capable of causing oxidative damage to lipids, proteins and nucleic acids (see, for example, Lai and Piette, in Biochem. Biophys. Res. Commun. 78:51-59 (1977); and Dizdaroglu and Bergtold, in Anal. Biochem., 156:182 (1986)).

The occurrence of iron imbalance resulting in excessive in vivo iron levels can be categorized into two conditions, namely iron-overload and non-iron overload conditions (see, for example, Voest et al., supra; Kontoghiorghes, supra). Iron-overload conditions are common in such patients as those suffering from thalassemia, sickle cell anemia, repeated blood transfusion and hereditary hemochromatosis. In such patients, transferrin is fully saturated with iron, and excess low-molecular-weight iron appears in the serum. This low-molecular-weight iron appears to originate from the iron released mainly from the liver and spleen, and from the breakdown of effete red cells. Other iron overload diseases and conditions include hereditary spherocytosis; hemodialysis, dietary or latrogenic iron intake, intramuscular iron dextran and hemolytic disease of the newborn (see, for example, Voest et al., supra; Kontoghiorghes, supra).

Non-iron overload conditions relate to situations where elevated iron levels are the result of therapeutic intervention, such as, for example, anthracycline anti-cancer therapy or inflammatory diseases such as rheumatoid arthritis. While anthracyclines such as adriamycin (doxorubicin) are effective in the treatment of a number of neoplastic diseases, these compounds have limited clinical utility due to the high incidence of cardiomyopathy (see, for example, Singal et al., in J. Mol. Cell. Cardiol. 19:817-828 (1987)).

The molecular mechanism of cardiomyopathy is now attributed to the adriamycin-induced release of iron from intracellular iron-containing proteins, resulting in the formation of an adriamycin-iron complex, which generates reactive oxygen species causing the scission and condensation of DNA, peroxidation of phospholipid membranes, depletion of cellular reducing equivalents,

interference with mitochondrial respiration, and disruption of cell calcium homeostasis (see, for example, Myers et al., Science 197:165-167 (1977); and Gianni et al., in Rev. Biochem. Toxicol. 5:1-82 (1983)). On the other hand, several clinical studies have shown that patients with rheumatoid arthritis exhibit elevated low-molecular weight iron species and ferritin-bound iron levels in synovial fluid. Iron, presumably via its mediation of oxygen free radical pathways, exerts its proinflammatory effects in rheumatoid arthritis (see, for example, Muirden and Senator, in Ann. Rheum. Dis. 27:38-48 (1968); and Biemond et al., in Arthritis Rheum. 29:1187-1193 (1986)).

Iron also plays an important role in many aspects of immune and nonimmune host response (see, for example, De Sousa et al., in Ann. N.Y. Acad. Sci. 526:310-323 (1988)). It is known that increased concentrations of iron are deleterious to the immune system through the initiation or maintenance of inflammatory reactions (see, for example, Biemond et al., in J. Clin. Invest. 73:1576-9 (1984); and Rowley et al., in Clin. Sci. 66:691-5 (1984)). Other non-iron overload diseases and conditions include reperfusion injury, solid tumors (e.g., neuroblastoma), hematologic cancers (e.g., acute myeloid leukemia), malaria, renal failure, Alzheimer's disease, Parkinson's disease, inflammation, heart disease, AIDS, liver disease (e.g., chronic hepatitis C), microbial/parasitic infections, myelofibrosis, drug-induced lung injury (e.g., paraquat), graft-versus-host disease and transplant rejection and preservation.

Hence, not surprisingly, there has been a tremendous interest in the therapeutic use of chelators in the treatment of both iron-overload and non-iron overload diseases and conditions. A chelator (Greek, chele-claw of a crab) is a molecule forming a cyclic ring with a metal as the closing member. Hundreds of chelating agents have been

designed and developed for animal and human studies. Among them, at least fifteen different chelators have been used in humans, including desferrioxamine (DF), ethylenediamine-tetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DTPA), pyridoxalisonicotinoylhydrazone (PIH), 1,2-dimethyl-3-hydroxypyrid-4-one (L1) and [+] 1,2-bis-(3,5-dioxopiperazine-1-yl) propane (ICRF-187).

For the past 30 years, DF (i.e., desferrioxamine) has been the most commonly used chelating drug for the treatment of transfusional iron overload (see, for example, Pippard et al., in Blood 60:288-294 (1982); Proper et al., in N. Engl. J. Med. 294:1421-1423 (1976); and St. Louis et al., in Lancet 336:1275-1279 (1990)). Patients suffering from thalassemia lived longer with the DF treatment. However, major drawbacks in the use of DF include the cost thereof (~$7,000/patient/year), which can be affordable only by a very small percentage of thalassemia patients worldwide. Another drawback to the use of DF includes the toxicity thereof, including ophthalmic and auditory toxicities as well as induction of pulmonary and renal damage.

Unlike DF, L1 (i.e., 1,2-dimethyl-3-hydroxypyrid-4-one) and related compounds are orally available iron chelators, showing promise in improving the quality of life in patients with thalassemia (see, for example, Olivieri et al., in Drugs Today 28(Suppl. A):123-132 (1992)) and rheumatoid arthritis (see, for example, Vreugdenhil et al., in Lancet 2:1398-9 (1989)). However, the major side effects of L1 therapy include myelosuppression, fatigue, and maternal, embryo and teratogenic toxicity, which severely limits the potential clinical applications thereof (see, for example, Kontoghiorghes, in Int. J. Hematol. 55:27-38 (1992)).

Recently, ICRF-187 has been demonstrated to be effective in removing iron from the anthracycline-iron complex, therefore preventing the cardiac toxicity in cancer patients receiving adriamycin chemotherapy (see, for example, Kolaric et al., in Oncology 52:251-5 (1995)). However, when chelated with iron, the iron-ICRF-187 complex per se is also very effective in the promotion of hydroxyl radical generation via the Fenton reaction, causing oxidative damage to tissues (see, for example, Thomas et al., in Biochem. Pharmacol. 45:1967-72 (1993)). In addition, since ICRF-187 is a strong chelator (having a structure similar to EDTA), it chelates not only low-molecular-weight iron, but also chelates iron from transferrin and ferritin, as well as copper from ceruloplasmin, thus potentially affecting normal cellular iron metabolism.

Chronic exposure of the skin to sunlight or ultraviolet radiation can cause severe damage to the underlying connective tissue, leading to erythema and other skin diseases (see, for example, Beisset and Granstein in Crit. Rev. Biochem. Mol. Biol. 31:381-404 (1995) and Kaminester in Arch. Fam. Med. 5:289 (1996). Although the mechanism by which photodamage occurs is not well understood, reactive oxygen species (such as singlet oxygen, superoxide and hydrogen peroxide) and reactive nitrogen species (such as nitric oxide and peroxynitrite) have been implicated as important contributors to such damage (see, for example, Jurkiewicz and Buettner in Photochem. Photobiol. 59:1-4 (1994), Deliconstantinos et al., in Biochem. Pharmacol. 51:1727-1738 (1996) and Deliconstantinos et al., in Brit. J. Pharmacol. 114:1257-1265 (1995)). The skin is known to contain high levels of iron (see, for example, Bissett et al., in Photochem. Photobiol. 54:215-223 (1991). Upon release intracellularly by ultraviolet radiation, iron can participate in oxygen radical formation, thus enhancing the likelihood of causing

photodamage, and enhancing the level of photodamage which actually occurs. For example, the combination topical application of the iron chelator, 2-furildioxime, in combination with sunscreen, has been shown to produce synergistic photoprotection (see, for example, Bissett et al., in J. Am. Acad. Dermatol. 35:546-549 (1991)). However, further development in the field is needed to produce more effective and safer iron chelators for the prevention of photoaging and photodamage.

Therefore, there is still a need in the art for a new class of iron chelators that are capable of removing free iron ions from body fluids, without affecting the normal cellular iron metabolism.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, methods have been developed for the *in vivo* reduction of free iron ion levels in a subject. The present invention employs a scavenging approach whereby free iron ions are bound *in vivo* to a suitable physiologically compatible scavenger, i.e., a compound capable of binding free iron ions. The resulting complex renders the free iron ions harmless, and is eventually excreted in the urine of the host. Further in accordance with the present invention, there have been developed compositions and formulations useful for carrying out the above-described methods.

An exemplary physiologically compatible scavenger contemplated for use in the practice of the present invention is a dithiocarbamate-based formulation. Dithiocarbamates according to the invention bind to free iron ions, forming a stable, water-soluble dithiocarbamate-iron complex. Dithiocarbamates are a class of low molecular-weight sulphur-containing compounds that are effective chelators (see, for example, Shinobu et al., in

Acta Pharmacol et Toxicol. 54:189-194 (1984)). For example, diethyldithiocarbamate (DETC) is used clinically for the treatment of nickel poisoning.

Dithiocarbamates, such as N-methyl-D-glucamine dithiocarbamate (MGD), chelate with ferrous or ferric iron to form a stable and water-soluble two-to-one $[(MGD)_2-Fe^{2+}]$ or $[(MGD)_2-Fe^{3+}]$ complex (see, for example, Lai and Komarov, in FEBS Letters 345:120-124 (1994)). However, MGD administrated into normal rats did not chelate endogenous iron to form the $[(MGD)_2-Fe]$ complex, suggesting that MGD does not remove iron from either hemoglobin or other iron containing enzymes or proteins. On the other hand, administration of MGD into endotoxin-treated rats resulted in the formation of the $[(MGD)_2-Fe]$ complex which could be detected in body fluids such as blood plasma and urine.

It is known that endotoxin challenge induces the release of cellular iron from tissues (see, for example, Kim et al., in J. Biol. Chem. 270:5710-5713 (1995)). Thus, dithiocarbamates such as MGD are capable of removing free iron *in vivo*, particularly during the infectious and inflammatory conditions where intracellular iron loss is common, therefore preventing iron-induced oxidative damage to the tissues. Additionally, MGD is safe inasmuch as injections of up to 1% of the body weight in rats did not produce any ill-effects (see, for example, Komarov and Lai, in Biochim. Biophys. Acta 1272:29-36 (1995)).

Another major complication in the therapeutic use of chelators is the propensity of chelators to affect not only the desired metal but also many other essential metals, their associated metabolic pathways and other processes. Thus, for example, the treatment with DF and L1 requires zinc supplementation to prevent the occurrence of zinc deficiency diseases (see, for example, De Virgilis et

al., Arch. Dis. Chil. 63:250-255 (1988); and Al-Refai et al., in Blood 80:593-599 (1992)).

The low-molecular-weight iron pool in serum is thought to be the most labile iron source during chelation therapy. Chelators that remove this low-molecular-weight iron with only a minimal effect on other essential metal contents in the body are highly desirable, particularly for the treatment of transfusion-induced iron overload, as well as iron overload induced by anthracycline anti-cancer agents, inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, and the like.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 provides UV-visible spectra of N-methyl-D-glucamine dithiocarbamate (MGD) and [MGD-Fe] complexes in aqueous solution.

Figure 1A provides a spectrum of MGD alone. An aliquot (10 μl) of MGD (100 mM) in water was added to 2 ml of water. Water was used as the control. The spectrum was recorded from 800 nm to 200 nm. Note that MGD showed an intensive absorption in the 200-300 nm range.

Figure 1B provides a spectrum of the [MGD-Fe] complex. An aliquot (40 μl) of ferrous sulfate (10 mM) was added to 2 ml of a 0.5 mM MGD solution in water. An MGD solution (0.5 mM) without ferrous sulfate was used as the control. Note the appearance of a prominent charge transfer band at 508 nm, indicative of the formation of an iron-chelator complex.

Figure 2 illustrates the time dependent changes of visible spectra of [MGD-Fe] complex. Thus, an MGD solution (25 mM) in water was purged with a stream of nitrogen gas for 15 min prior to addition of an aliquot of

nitrogen-saturated ferrous sulfate solution in water to a final concentration of 5 mM. The superimposed spectra were obtained by repetitive scanning using a three-min scan time. Other spectrometer settings included scan speed 100 nm/min and chart speed 25 nm/cm. Note that the charge transfer peak at 508 nm increased with time, indicating the autoxidation of ferrous iron to ferric iron in the [MGD-Fe] complex.

Figure 3 presents the results of titration experiments on the complexation between MGD and Fe^{3+}. Serial titration experiments were performed to determine the binding stoichiometry between MGD and Fe^{3+}. The MGD solution in aerated water was kept at a constant concentration of 0.5 mM to which was added various amounts of ferrous sulfate (Fe^{2+}) from 0.05 mM to 0.5 mM with a tenth increment. The mixtures of MGD and Fe^{2+} were incubated at 22°C for 10 min to allow the time required for reaching an equilibrium between the [MGD-Fe^{2+}] and [MGD-Fe^{3+}] complexes. The O.D. at 508 nm was plotted against the Fe/MGD ratios.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for the <u>in vivo</u> reduction of free iron ion levels in a subject. Invention methods comprise:

administering to a subject an effective amount of at least one physiologically compatible compound capable of binding free iron ions.

Exemplary physiologically compatible compounds contemplated for use in the practice of the present invention are dithiocarbamates. These materials are said to be "physiologically compatible" because they do not induce any significant side effects. In other words, the

main effect exerted by these compounds is to bind free iron ions.

As used herein, the phrase "free iron ions" refers to transient iron species which are not stably incorporated into a biological complex (e.g., hemoglobin, ferritin, and the like). Scavengers contemplated for use herein are highly selective for "free iron ions", relative to other forms of iron present in a physiological system.

Dithiocarbamate compounds contemplated for use in the practice of the present invention include any physiologically compatible derivative of the dithiocarbamate moiety (i.e., $(R)_2N\text{-}C(S)\text{-}SH$). Such compounds can be described with reference to the following generic structure:

$$[R_1R_2N\text{-}C(S)\text{-}S^-]\ M^{+1 \text{ or } +2} \qquad \text{(I)}$$

wherein:

each of R_1 and R_2 is independently selected from a C_1 up to C_{18} alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclic, substituted heterocyclic, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, aroyl, substituted aroyl, acyl, substituted acyl, or

R_1 and R_2 can cooperate to form a 5-, 6- or 7-membered ring including N, R_1 and R_2, or

R_1 or R_2 is a divalent moiety selected from the group consisting of alkylene, substituted alkylene, oxyalkylene, substituted oxyalkylene, alkenylene, substituted

alkenylene, arylene, substituted arylene, alkarylene, substituted alkarylene, aralkylene, substituted aralkylene, aralkenylene, substituted aralkenylene, aralkynylene, substituted aralkynylene, cycloalkylene, substituted cycloalkylene, heterocycloalkylene or substituted heterocycloalkylene, wherein said divalent moiety serves as the same substituent for two dithiocarbamate structures, thereby linking said structures together so as to form a bis(dithiocarbamate) species, and

M is a monovalent or divalent cation.

Monovalent cations contemplated for incorporation into the above-described dithiocarbamate compounds include H^+, Na^+, NH_4^+, tetraalkyl ammonium, and the like. Divalent cations contemplated for incorporation into the above-described dithiocarbamate compounds include zinc, calcium, magnesium, manganese, and the like (e.g., Zn^{+2}, Ca^{+2}, Mg^{+2}, or Mn^{+2}). In accordance with the present invention, the ratio of dithiocarbamate-species to counter-ion M can vary widely. Thus, dithiocarbamate-containing iron scavenger can be administered without any added metallic counter-ion (i.e., $M = H^+$, or a metal cation to dithiocarbamate-species ratio of zero), with ratios of metal cation to dithiocarbamate-species up to about 1:2 (i.e., a 2:1 dithiocarbamate:metal cation complex) being suitable.

Presently preferred compounds having the above-described generic structure are those wherein:

each of R_1 and R_2 = a C_1 up to C_{12} alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl, wherein the substituents are selected from carboxyl, -C(O)H, oxyacyl, phenol, phenoxy, pyridinyl,

pyrrolidinyl, amino, amido, hydroxy, nitro or sulfuryl, or

R_1 or R_2 is a divalent moiety selected from the group consisting of alkylene, substituted alkylene, oxyalkylene, substituted oxyalkylene, alkenylene, substituted alkenylene, arylene, substituted arylene, alkarylene, substituted alkarylene, aralkylene, substituted aralkylene, aralkenylene, substituted aralkenylene, aralkynylene, substituted aralkynylene, cycloalkylene, substituted cycloalkylene, heterocycloalkylene or substituted heterocycloalkylene, wherein said divalent moiety serves as the same substituent for two dithiocarbamate structures, thereby linking said structures together so as to form a bis(dithiocarbamate) species, and

$M = H^+, Na^+, Zn^{+2}, Ca^{+2}$ or Mg^{+2}.

Especially preferred compounds having the above-described generic structure are those wherein:

R_1 = a C_2 up to C_{10} alkyl or substituted alkyl, wherein the substituents are selected from carboxyl, acetyl, pyridinyl, pyrrolidinyl, amino, amido, hydroxy or nitro,

R_2 is selected from a C_1 up to C_6 alkyl or substituted alkyl, or R_2 can cooperate with R_1 to form a 5-, 6- or 7-membered ring including N, R_2 and R_1, or

R_1 or R_2 is a divalent moiety selected from the group consisting of alkylene, substituted alkylene, oxyalkylene,

substituted oxyalkylene, alkenylene, substituted alkenylene, arylene, substituted arylene, alkarylene, substituted alkarylene, aralkylene, substituted aralkylene, aralkenylene, substituted aralkenylene, aralkynylene, substituted aralkynylene, cycloalkylene, substituted cycloalkylene, heterocycloalkylene or substituted heterocycloalkylene, wherein said divalent moiety serves as the same substituent for two dithiocarbamate structures, thereby linking said structures together so as to form a bis(dithiocarbamate) species, and

$M = H^+$, Na^+, Zn^{+2}, Ca^{+2} or Mg^{+2}.

The presently most preferred compounds having the above-described generic structure are those wherein:

R_1 = a C_2 up to C_8 alkyl or substituted alkyl, wherein the substituents are selected from carboxyl, acetyl, amido or hydroxy,

R_2 = a C_1 up to C_4 alkyl or substituted alkyl, or

R_1 or R_2 is a divalent moiety selected from the group consisting of alkylene, substituted alkylene, oxyalkylene, substituted oxyalkylene, cycloalkylene, substituted cycloalkylene, heterocycloalkylene or substituted heterocycloalkylene having in the range of about 4 up to 11 carbon atoms, wherein said divalent moiety serves as the same substituent for two dithiocarbamate structures, thereby

linking said structures together so as to form a bis(dithiocarbamate) species, and

$M = H^+, Na^+, Zn^{+2}, Ca^{+2}$ or Mg^{+2}.

When R_1 and R_2 cooperate to form a 5-, 6- or 7-membered ring, the combination of R_1 and R_2 can be a variety of saturated or unsaturated 4, 5 or 6 atom bridging species selected from alkenylene or -O-, -S-,-C(O)- and/or -N(R)-containing alkylene moieties, wherein R is hydrogen or a lower alkyl moiety.

As employed herein, "substituted alkyl" comprises alkyl groups further bearing one or more substituents selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), cycloalkyl, substituted cycloalkyl, heterocyclic, substituted heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, aryloxy, substituted aryloxy, halogen, trifluoromethyl, cyano, nitro, nitrone, amino, amido, ester, -C(O)H, acyl, oxyacyl, carboxyl, carbamate, sulfonyl, sulfonamide, sulfuryl, and the like.

As employed herein, "cycloalkyl" refers to cyclic ring-containing groups containing in the range of about 3 up to 8 carbon atoms, and "substituted cycloalkyl" refers to cycloalkyl groups further bearing one or more substituents as set forth above.

As employed herein, "alkenyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and having in the range of about 2 up to 12 carbon atoms, and "substituted alkenyl" refers to alkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "alkynyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon triple bond, and having in the range of about 2 up to 12 carbon atoms, and "substituted alkynyl" refers to alkynyl groups further bearing one or more substituents as set forth above.

As employed herein, "aryl" refers to aromatic groups having in the range of 6 up to 14 carbon atoms and "substituted aryl" refers to aryl groups further bearing one or more substituents as set forth above.

As employed herein, "alkylaryl" refers to alkyl-substituted aryl groups having in the range of about 7 up to 16 carbon atoms and "substituted alkylaryl" refers to alkylaryl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkyl" refers to aryl-substituted alkyl groups having in the range of about 7 up to 16 carbon atoms and "substituted arylalkyl" refers to arylalkyl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkenyl" refers to aryl-substituted alkenyl groups having in the range of about 8 up to 16 carbon atoms and "substituted arylalkenyl" refers to arylalkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkynyl" refers to aryl-substituted alkynyl groups having in the range of about 8 up to 16 carbon atoms and "substituted arylalkynyl" refers to arylalkynyl groups further bearing one or more substituents as set forth above.

As employed herein, "aroyl" refers to aryl-carbonyl species such as benzoyl and "substituted aroyl" refers to aroyl groups further bearing one or more substituents as set forth above.

As employed herein, "heterocyclic" refers to cyclic (i.e., ring-containing) groups containing one or more heteroatoms (e.g., N, O, S, or the like) as part of the ring structure, and having in the range of 3 up to 14 carbon atoms and "substituted heterocyclic" refers to heterocyclic groups further bearing one or more substituents as set forth above.

As employed herein, "acyl" refers to alkyl-carbonyl species.

As employed herein, "halogen" refers to fluoride, chloride, bromide or iodide atoms.

As employed herein, "alkylene" refers to saturated, divalent straight or branched chain hydrocarbyl groups typically having in the range of about 2 up to 12 carbon atoms, and "substituted alkylene" refers to alkylene groups further bearing one or more substituents as set forth above.

As employed herein, "oxyalkylene" refers to saturated, divalent straight or branched chain hydrocarbyloxy groups typically having in the range of about 1 up to 12 carbon atoms, and "substituted oxyalkylene" refers to oxyalkylene groups further bearing one or more substituents as set forth above.

As employed herein, "alkenylene" refers to divalent straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and typically having in the range of about 2 up to 12 carbon

atoms, and "substituted alkenylene" refers to alkenylene groups further bearing one or more substituents as set forth above.

As employed herein, "arylene" refers to divalent aromatic groups typically having in the range of 6 up to 14 carbon atoms and "substituted arylene" refers to arylene groups further bearing one or more substituents as set forth above.

As employed herein, "alkarylene" refers to alkyl-substituted divalent aryl groups typically having in the range of about 7 up to 16 carbon atoms and "substituted alkarylene" refers to alkarylene groups further bearing one or more substituents as set forth above.

As employed herein, "aralkylene" refers to aryl-substituted divalent alkyl groups typically having in the range of about 7 up to 16 carbon atoms and "substituted aralkylene" refers to aralkylene groups further bearing one or more substituents as set forth above.

As employed herein, "aralkenylene" refers to aryl-substituted divalent alkenyl groups typically having in the range of about 8 up to 16 carbon atoms and "substituted aralkenylene" refers to aralkenylene groups further bearing one or more substituents as set forth above.

As employed herein, "aralkynylene" refers to aryl-substituted divalent alkynyl groups typically having in the range of about 8 up to 16 carbon atoms and "substituted aralkynylene" refers to aralkynylene groups further bearing one or more substituents as set forth above.

As employed herein, "cycloalkylene" refers to divalent ring-containing groups containing in the range of about 3 up to 8 carbon atoms, and "substituted cycloalkylene" refers to cycloalkylene groups further bearing one or more substituents as set forth above.

As employed herein, "heterocycloalkylene" refers to divalent cyclic (i.e., ring-containing) groups containing one or more heteroatoms (e.g., N, O, S, or the like) as part of the ring structure, and having in the range of 3 up to 14 carbon atoms and "substituted heterocycloalkylene" refers to heterocycloalkylene groups further bearing one or more substituents as set forth above.

In accordance with another embodiment of the present invention, there are provided methods for treating subjects having elevated circulating levels of free iron ions. Invention methods comprise:

administering to a subject an effective amount of at least one physiologically compatible compound capable of binding free iron ions.

In accordance with yet another embodiment of the present invention, there are provided methods for treating overproduction of free iron ions in a subject. Invention methods comprise:

administering to a subject an effective amount of at least one physiologically compatible compound capable of binding free iron ions.

The presence of elevated iron levels in a subject is associated with a wide range of disease states and/or indications, such as, for example, hereditary conditions (e.g., thalassemia, sickle cell anemia, hereditary hemochromatosis, hereditary spherocytosis, hemolytic disease of the newborn, and the like), afflictions related

to invasive exchange of body fluids (e.g., repeated blood transfusions, hemodialysis, cardiopulmonary bypass, ischemic/reperfusion injury, dietary iron uptake, latrogenic iron uptake, intramuscular iron dextran, and the like).

Additional indications associated with elevated levels of free iron ions include anthracycline anti-cancer therapy, inflammation (e.g., liver inflammation, renal inflammation, and the like), , septic shock, hemorrhagic shock, anaphylactic shock, toxic shock syndrome, arthritis (e.g., rheumatoid arthritis), ulcers, ulcerative colitis, inflammatory bowel disease, gastritis, adult respiratory distress syndrome, asthma, cachexia, transplant rejection, myocarditis, multiple sclerosis, diabetes mellitus, autoimmune disorders, eczema, psoriasis, glomerulonephritis, heart failure, heart disease, atherosclerosis, Crohn's disease, dermatitis, urticaria, ischemia, cerebral ischemia, systemic lupus erythematosis, AIDS, AIDS dementia, chronic neurodegenerative disease, chronic pain, priapism, cystic fibrosis, amyotrophic lateral sclerosis, schizophrenia, depression, premenstrual syndrome, anxiety, addiction, migraine, Parkinson's disease, Huntington's disease, epilepsy, neurodegenerative disorders, gastrointestinal motility disorders, obesity, hyperphagia, ischemia/reperfusion injury, allograft rejection, solid tumors (e.g., neuroblastoma), malaria, cancers (e.g., breast, melanoma, carcinoma, hematologic cancers, and the like), Alzheimer's disease, infection (including bacterial, viral, fungal and parasitic infections), myelofibrosis, lung injury, graft-versus-host disease, head injury, CNS trauma, cirrhosis, hepatitis, renal failure, liver disease (e.g., chronic hepatitis C), drug-induced lung injury (e.g., paraquat), transplant rejection and preservation, burn, administration of cytokines, overexpression of cytokines, encephalomyelitis, meningitis, pancreatitis, peritonitis, vasculitis,

lymphocytic choriomeningitis, uveitis, ileitis, myasthenia gravis (MG), ophthalmic diseases, post-angioplasty, restenosis, angina, coronary artery disease, stroke, chronic fatigue syndrome, photoaging, photodamage, and the like.

With particular reference to cytokine therapy, the invention method will find widespread use because cytokine therapy (with consequent induction of release of free iron ions) is commonly used in the treatment of cancer and AIDS patients. Side effects due to the induction of free iron ion release are problems commonly associated with cytokine therapy (see, for example, Lissoni et al in J. Biol. Regulators Hemeostatic Agents 7:31-33 (1993)). Thus, a large patient population exists which will benefit from invention methods.

Presently preferred indications for treatment in accordance with the present invention include administration of interleukin-1 (IL-1), administration of interleukin-2 (IL-2), administration of interleukin-6 (IL-6), administration of interleukin-11 (IL-11), administration of interleukin-12 (IL-12), administration of tumor necrosis factor (TNF), administration of interferon-alpha (IF-α) or interferon-gamma (IF-γ), arthritis, asthma, Alzheimer's disease, Parkinson's disease, multiple sclerosis, cirrhosis or allograft rejection. Especially preferred indications for treatment in accordance with the present invention include release of free iron ions associated with cytokine therapy.

As readily understood by those of skill in the art, a wide variety of agents and/or conditions induce release of free iron ions. Thus, invention compositions can advantageously be included in combination with treating agents for such indications. Thus, for example, invention compositions can be combined with anti-inflammatory agents,

immunosuppressants, antistroke agents, anticancer agents, thrombolytic agents, neuroprotectants, nitric oxide synthase inhibitors, anti-migraine agents, and the like.

Examplary treatments for which the above-described combinational therapy employing invention compositions is contemplated include:

inflammatory disease therapy (e.g., employing disease-modifying agents (such as antimalarials, methotrexate, sulfasalazine, mesalamine, azathioprine, 6-mercaptopurine, metronidazole, injectable and oral gold, D-penicillamine, and the like), corticosteroids, non-steroidal antiinflammatory drugs (such as acetominophen, aspirin, sodium salicylate, magnesium salicylate, choline magnesium salicylate, salicylsalicylic acid, ibuprofen, naproxen, diclofenac, diflunisal, etodolac, fenoprofen calcium, fluriprofen, piroxicam, indomethacin, ketoprofen, ketorolac tromethamine, meclofenamate, meclofenamate sodium, mefenamic acid, nabumetone, oxaprozin, phenyl butyl nitrone (PBN), sulindac, tolmetin, and the like), and the like),

immunosuppression (e.g., employing one or more agents such as cyclosporin A, OKT3, FK506, mycophenolate mofetil (MMF), azathioprine, corticosteroids (such as prednisone), antilymphocyte globulin, antithymocyte globulin, and the like),

stroke therapy (e.g., employing one or more agents such as fibrinolytic agents (such as streptokinase, acylated plasminogen-streptokinase complex, urokinase, tissue plasminogen activator, and the like),

employing monoclonal antibodies directed against leukocyte adhesion molecules (such as intercellular adhesion molecule-1 (ICAM-1), CD18, and the like), hemodilution therapy (employing modified hemoglobin solutions such as diaspirin crosslinked hemoglobin), employing growth factors (such as basic fibroblast growth factor (bFGF), transforming growth factor-beta 1 (TGF-ß1), and the like), employing glutamate antagonists (such as lamotrigine, dizolcilpine maleate (MK 801), BW619C89, BW1003C87, and the like), employing NMDA antagonists (such as CGS 19755 (Selfotel), aptiganel hydrochloride, dextrorphar, d-CPPene, and the like), employing GABA agonists (such as muscimol), employing free radical scavengers (such as allopurinol, S-PBN, 21-aminosteroids, tocopherol, superoxide dismutase, dexanabinol (HU-211), selenium, carotenoids, and the like), idebenone, ticlopidine, lovastatin, citicoline, and the like),

anti-cancer therapy (e.g., employing one or more agents such as alkylating agents (such as mechlorethamine, chlorambuccil, ifosfamide, melphalan, busulfan, carmustine, lomustine, procarbazine, dacarbazine, cisplatin, carboplatin, and the like), antimetabolites (such as methotrexate, mercaptopurine, thioguanine fluorouracil, cytarabine, and the like), hormonal agents (such as testosterone propionate, fluoxymesterone, flutamide, diethylstilbestrol, ethinyl estradiol, tamoxifen, hydroxyprogesterone caproate, medroxyprogesterone, megestrol acetate, and the like),

adrenocorticosteroids (such as prednisone), aromatase inhibitors (such as amino glutethimide), leuprolide, goserelin acetate, biological response modifiers (such as interferon-α2a, interferon-α2b, interleukin-2, and the like), peptide hormone inhibitors (such as octreotide acetate), natural products (such as vinblastine, vincristine, vinorelbine, paclitaxel, dactinomycin, daunorubicin, idarubicin, doxorubicin, etoposide, plicamycin, mitomycin, mitoxantrone, bleomycin, hydroxyurea, mitotane, fludarabine, cladribine, and the like), supportive agents (such as allopurinol, mesna, leucovorin, erythropoietin, filgrastim, sargramostim, and the like), and the like,

thrombolytic therapy for acute myocardial infarction (e.g., employing agents such as streptokinase, tissue plasminogen activator (t-PA), anistreplase, and the like),

administration of neuroprotective agents, such as α-adrenoreceptor antagonist (e.g., α-dihydroergocryptine), NMDA antagonists (e.g., remacemide, 2-piperazinecarboxylic acid, N-indologlycinamide derivatives, spiro[benzo(b)thiophen-4(5H)] derivatives, eliprodil, dexanabinol, amantadine derivatives, dizocilpine, benzomorphan derivatives, aptiganel, (S)-α-phenyl-2-pyridine ethanamide dihyrochloride, 1-amino-cyclopentanecarboxylic acid, and the like), sodium channel antagonists, glycine antagonists (e.g., glystasins), calcium channel antagonists (e.g., 3,5-pyridinedicarboxylic acid derivatives,

conopeptides, 1-piperazineethanol, thieno[2,3-b]pyridine-5-carboxylic acid derivatives, nilvadipine, nisoldipine, tirilazad mesylate, 2H-1-enzopyran-6-ol, nitrone spin traps, iacidipine, iomeerzine hydrochloride, lemildipine, lifarizine, efonidipine, piperazine derivatives, and the like), calpain inhibitors, fibrinogen antagonists (e.g., ancrod), integrin antagonists (e.g., antegren), thromboxane A_2 antagonist (e.g., 9H-carbazole-9-propanoic acid derivatives, 5-Heptenoic acid derivatives, 1-azulene-sulfonic acid derivatives, and the like), brain-derived neurotropic factor, adrenergic transmitter uptake inhibitor (e.g., 1-butanamine), endothelin A receptor antagonists (e.g., benzenesulfonamide derivatives), GABA A receptor antagonists (e.g., triazolopyrimidine derivatives, cyclohexaneacetic acid derivatives, and the like), GPIIb IIIa receptor antagonists, platelet aggregation antagonist (e.g., 2(1H)-quinolinone derivatives, 1H-pyrrole-1-acetic acid derivatives, coumadin, and the like), Factor Xa inhibitor, corticotropin releasing factor agonist, thrombin inhibitor (e.g., fraxiparine, dermatan sulfate, heparinoid, and the like), dotarizine, intracellular calcium chelators (e.g., BAPTA derivatives), radical formation antagonists (e.g., EPC-K1, 3-pyridinecarboxamide derivatives, superoxide dismutase, raxofelast, lubeluzole, 3H-pyrazol-3-one derivatives, kynurenic acid derivatives, homopiperazine derivatives, polynitroxyl albumin, and the like), protein kinase

inhibitors (e.g., 1H-1,4-diazepine), nerve growth agonist, glutamate antagonist (e.g., cyclohexanepropanoic acid, riluzole, acetamide derivatives, and the like), lipid peroxidase inhibitors (e.g., 2,5-cyclohexadiene-1,4-dione derivatives), sigma receptor agonist (e.g., cyclopropanemethanamine derivatives), thyrotropin releasing hormone agonist (e.g., L-prolinamide, posatirelin, and the like), prolyl endopeptidase inhibitor, monosialoganglioside GM1, proteolytic enzyme inhibitor (e.g., nafamostat), neutrophil inhibitory factor, platelet activating factor antagonist (e.g., nupafant), monoamine oxidase B inhibitor (e.g., parafluoroselegiline, benzonitrile derivatives, and the like), PARS inhibitors, Angiotensin I converting enzyme inhibitor (e.g., perindopril, ramipril, and the like), acetylcholine agonist (e.g., pramiracetam), protein systhesis antagonist (e.g., procysteine), phosphodiesterase inhibitor (e.g., propentofylline), opioid kappa receptor agonist (e.g., 10H-phenothiazine-2-carboxamine derivatives), somatomedin-1, carnitine acetyltransferase stimulant (e.g., acetylcarnitine), and the like.

inhibition of nitric oxide synthase enzymes (e.g., employing arginine analogs (such as L-N^G-methylarginine, L-N^G-nitroarginine, L-N^G-aminoarginine, L-iminoethylornithine, ϵ-N-iminoethyl-L-lysine, L-N^G-nitroarginine methyl ester, L-N^G-hydroxyl-N^G-methylarginine, L-N^G-methyl-N^G-methylarginine, L-thiocitrulline, L-S-methylthiocitrulline,

L-S-ethylisothiocitrulline, S-ethylisothiocitrulline, aminoguanidine, S-methyl isothiourea sulfate, and the like), heme ligands (such as 7-nitroindazole, 7,7,8,8-tetramethyl-o-quinodimethane, imidazole, 1-phenylimidazole, 2-phenylimidazole, and the like), calmodulin antagonists (such as chlorpromazine, W-7, and the like), and the like);

administration of antimigraine agents, such as naratriptan, zolmitriptan, rizatriptan, quetiapine, Phytomedicine, (S)-fluoxetine, calcium channel antagonists (e.g., nimodipine/Nimotop, flunarizine, dotarizine, iomerizine HCl, and the like), α-dihydroergocryptine, 5-HT1 agonists, (e.g., Sumatriptan/Imitrex, Imigran, and the like), 5-HT1D agonists, 5-HT1A antagonists, 5-HT1B antagonists, 5-HT1D antagonists (e.g., 1H-indole-5-ethanesulfonamide derivatives, 1H-indole-5-methanesulfonamide, and the like), 2-thiophenecarboxamide, 3-piperidinamine, diclofenac potassium, dihydroergotamine, dolasetron mesilate, dotarizine, flupirtine, histamine-H3 receptor agonist, indobufen, 1-azulenesulfonic acid derivatives, cholinesterase inhibitors, bradykinin antagonists, substance P antagonists (e.g., Capsaicin/Nasocap), piperazine derivatives, neurokinin 1 antagonists, metergoline, dopamine D2 antagonist (e.g., metoclopramide + lysine acetyl), enkephalinase inhibitors (e.g., neutral endopeptidase), 5-HT2 antagonists, 5-HT3 antagonists (e.g., Dolasetron mesilate, 4H-carbazol-4-one derivatives, and the like), tenosal,

tolfenamic acid, cyclooxygenase inhibitors (e.g., carbasalate/carbaspirin calcium, tenosal, and the like), alpha adrenoreceptor antagonists (e.g., arotinolol, dihydroergocryptine, and the like), opioid agonists (e.g., flupirtine), beta adrenergic antagonists (e.g., propranolol), valproate semisodium, and the like.

In accordance with a particular aspect of the present invention, the dithiocarbamate-containing iron scavenger is administered in combination with a cytokine (e.g., IL-1, IL-2, IL-6, IL-11, IL-12, TNF or interferon-γ), an antibiotic (e.g., gentamicin, tobramycin, amikacin, piperacillin, clindamycin, cefoxitin or vancomycin, or mixtures thereof), a vasoactive agent (e.g., a catecholamine, noradrenaline, dopamine or dobutamine), or mixtures thereof. In this way, the detrimental side effects of many of the above-noted pharmaceutical agents (e.g., induction of release of free iron ions) can be prevented or reduced by the dithiocarbamate-containing scavenger. Thus, a patient being treated with any of the above-described agents could be monitored for evidence of elevated free iron ion levels. At the first evidence of such elevated levels of free iron ions, co-administration of a suitable dose of the above-described dithiocarbamate-containing scavenger could be initiated, thereby alleviating (or dramatically reducing) the side-effects of the primary therapy.

Those of skill in the art recognize that the dithiocarbamate-containing scavengers described herein can be delivered in a variety of ways, such as, for example, orally, topically (i.e., for cosmetic as well as therapeutic purposes), intravenously, subcutaneously, parenterally, rectally, nasally (i.e., by inhalation), and the like.

Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, the precise mode of administration and dosage employed for each subject is left to the discretion of the practitioner. In general, the dosage of dithiocarbamate-containing scavengers employed in the practice of the present invention falls in the range of about 5 mg - 18.5 g/day. Presently preferred modes of administration are oral, topical, by inhalation or by injection.

In accordance with still another embodiment of the present invention, there are provided physiologically active composition(s) comprising a compound having the structure **I**, as described above, in a suitable vehicle rendering said compound amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, and the like. Depending on the mode of delivery employed, the dithiocarbamate-containing scavenger can be delivered in a variety of pharmaceutically (and/or cosmetically) acceptable forms. For example, the scavenger can be delivered in the form of a solid, solution, emulsion, dispersion, micelle, liposome, and the like.

Pharmaceutically (and/or cosmetically) acceptable compositions of the present invention can be used in the form of a solid, a solution, an emulsion, a dispersion, a micelle, a liposome, and the like, wherein the resulting composition contains one or more of the compounds of the present invention, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for topical, enteral or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically (and/or cosmetically) acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, and any

other form suitable for use. The carriers which can be used include glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, medium chain length triglycerides, dextrans, and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form. In addition auxiliary, stabilizing, thickening and coloring agents and perfumes may be used. The active compound (i.e., compounds of structure I as described herein) is included in the pharmaceutically (and/or cosmetically) acceptable composition in an amount sufficient to produce the desired effect upon the process or condition of diseases.

Pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of a sweetening agent such as sucrose, lactose, or saccharin, flavoring agents such as peppermint, oil of wintergreen or cherry, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients may also be manufactured by known methods. The excipients used may be, for example, (1) inert diluents such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents such as corn starch, potato starch or alginic acid; (3) binding agents such as gum tragacanth, corn starch, gelatin or acacia, and (4) lubricating agents such as magnesium stearate, stearic acid or talc. The

tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate or bioadhesive polymers (or mucoadhesive polymers) may be employed. They may also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release.

In some cases, formulations for oral use may be in the form of hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

The pharmaceutical compositions may be in the form of a sterile injectable suspension. This suspension may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides, fatty acids (including oleic acid), naturally occurring vegetable oils like sesame oil, coconut oil, peanut oil, cottonseed oil, etc., or synthetic fatty vehicles like ethyl oleate or the like. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

Compounds contemplated for use in the practice of the present invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters of polyethylene glycols, which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the drug.

Compounds contemplated for use in the practice of the present invention may also be formulated for topical administration, for example, as a skin lotion, suntan lotion, cosmetic lotion, moisturizer, lip balm, eye makeup, face cream, and the like. A typical formulation includes one or more compounds as described herein, in combination with moisturizers, antioxidants, and the like.

Moisturizers contemplated for use in the above-described topical formulations include occlusive moisturizers, such as, for example, hydrocarbon oils and waxes, petroleum jelly, silicone oils, silicone derivatives, vegetable and animal fats, cocoa butter, mineral oil, fatty acids, fatty alcohols, lanolin, phospholipids, and the like; humectants, such as, for example, glycerin, honey, lactic acid, sodium lactate, ceramide, urea, propylene glycol, sorbitol, pyrrolidone carboxylic acid, glycolic acid, gelatin, vitamins, proteins, and the like; hydrophilic matrices, such as, for example, hyaluronic acid, colloidal oatmeal, and the like; essential fatty acids (e.g., Dermasil), elastin, niosomes, and the like.

Antioxidants contemplated for use in the above-described topical formulations include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, γ-tocopherol, α-tocopherol, ubiquinol 10,

ubiquinone 10, ascorbic acid, uric acid, glutathione, and the like.

Commonly used active ingredients in sunscreen products include para-aminobenzoic acid (PABA), benzophenone, padimate O, cinnamates, homosalate, oxybenzone, octylsalicylates, and the like. Exemplary sunscreen products include Shade SPF15 (available from Schering-Plough Corp., Memphis, TN), Pre-Sun SPF15 cream (available from Westwood-Bristol Myers, Buffalo, NY), Sundown SPF15 (available from Proctor and Gamble, Cincinnati, OH), Bullfrog SPF36 (available from Chattem, Inc., Chattanooga, TN), Daylong 16 (available from SpirigAG, CH-Egerkingen, an emulsion gel containing 70% water, ethanol, phospholipids, carbopol, sorbitol, silicone, amphisol, cetyl alcohol, tocopherol, triethanolamine, preservatives, and preparations with white petroleum jelly as vehicle, and the like.

Commonly used active ingredients in skin care products include alpha-hydroxy acids, tocopherol sorbate, ascorbate, glycolic acid, and the like.

Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, it is up to the practitioner to determine a subject's response to treatment and vary the dosages accordingly.

Typical daily doses, in general, lie within the range of from about 80 μg up to about 300 mg per kg body weight, and, preferably within the range of from 100 μg to 10 mg per kg body weight and can be administered up to four times daily. The typical daily IV dose lies within the range of from about 10 μg to about 100 mg per kg body weight, and, preferably, within the range of from 50 μg to 10 mg per kg body weight.

In accordance with yet another embodiment of the present invention, there are provided compositions comprising an anthracycline anti-cancer agent and a dithiocarbamate having the structure **I**, as described above.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1

UV-visible spectra of N-methyl-D-glucamine dithiocarbamate and MGD-Fe complex

N-methyl-D-glucamine dithiocarbamate synthesized by Shinobu et al's method (Shinobu et al., supra) was highly pure as determined by element analysis and by NMR. Figure 1A shows the UV-visible spectrum of MGD in water, which displays an intense absorption at uv regions and exhibited no absorption at the visible wavelength ranges. Addition of ferrous sulfate to the MGD solution under air-saturated conditions exhibits a red shift, with prominent absorption peaks appearing in the 300-400 nm range and a charge transfer band appearing at 508 nm, which is characteristic of the presence of the iron-chelator complex (Figure 1B).

Example 2

Autoxidation of the [MGD-Fe^{2+}] complex to the [MGD-Fe^{3+}] complex in aqueous solution

A mixture of MGD (25 mM) and Fe (5 mM) in aerated water exhibited a prominent charge transfer band at 508 nm, indicative of the presence of the [MGD-Fe^{3+}] complex. The [MGD-Fe^{2+}] complex (25 mM/5 mM) in water prepared under anaerobic conditions exhibited no absorption peak at 508 nm. However, when the above solution was exposed to the atmospheric oxygen, the visible regions of the absorption spectra changed with time as shown in Figure 2, in which a

charge transfer peak at 508 nm increased with time. This appearance of the absorption peak at 508 nm suggests the occurrence of the autoxidation of ferrous to ferric iron in the presence of oxygen. After 30 min incubation, about 25% of the [MGD-Fe^{2+}] complex was converted to the [MGD-Fe^{3+}] complex based on the calculation of the absorption intensity at 508 nm compared to that obtained from the known concentration of the [MGD-Fe^{3+}] complex. The results here suggest that [MGD-Fe^{2+}] can be autoxidated to [MGD-Fe^{3+}] and that MGD is capable of chelating either Fe^{2+} or Fe^{3+} ions forming stable complexes.

Example 3

Binding stoichiometry of the MGD-Fe complex

The binding stoichiometry between MGD and Fe was determined by varying concentrations of ferrous sulfate from 0.05 mM to 0.5 mM and by keeping a constant concentration of MGD (0.5 mM) in water. After 10 min incubation time, the O.D. at 508 nm of the samples were recorded and plotted as a function of the ratios of the Fe/MGD as shown in Figure 3. The extrapolation of the initial linear region showed the stoichiometry of Fe bound to MGD was 0.5 to 1. It is concluded that two MGD molecules bind one Fe to form a two-to-one [$(MGD)_2$-Fe] complex.

Example 4

In vivo chelation of endogenous iron by MGD in LPS-induced shock rats

As previously described (see Komarov and Lai in Biochim. Biophys Acta 1272:29-36 (1995), subcutaneous administration of the [$(MGD)_2$/Fe] complex reduced the *in vivo* ·NO levels in LPS-treated mice. Since excessive ·NO production is known to induce systemic hypotension, injections of the [$(MGD)_2$/Fe] complex that reduce *in vivo*

·NO levels should also restore blood pressure in hypotensive animals induced by LPS treatment. To test this idea, experiments were carried out to determine the effects of administration of the $[(MGD)_2/Fe]$ complex on the blood pressure of the hypotensive rats induced by LPS challenge.

Thus, male Wistar rats (230-300 g) fasted overnight were anesthetized with thiobutabarbital (Inactin, 100 mg/kg, i.p.). A catheter was implanted in the femoral vein for drug infusions. The femoral artery was cannulated for continuous blood pressure measurement. Rats were injected with an i.v. bolus dose of LPS (S.Typhosa endotoxin, 4 mg/kg). Two hours after LPS challenge, rats were then subjected to one of the following treatments:

(a) Control, saline infusion- 1.0 ml saline i.v. injection followed by 1.0 ml/hr of saline infusion for 1.5 hours,

(b) $[(MGD)_2/Fe]$ (at a ratio of 2-to-0.4)-0.1 mmole/kg i.v. bolus injection followed by 0.1 mmole/kg infusion for 1.5 hours,

(c) $[(MGD)_2/Fe]$ (at a ratio of 2-to-0.2)-0.1 mmole/kg i.v. bolus injection followed by 0.1 mmole/kg infusion for 1.5 hours, and

(d) $[(MGD)_2/Fe]$ (at a ratio of 2-to-0)-0.1 mmole/kg i.v. bolus injection followed by 0.1 mmole/kg infusion for 1.5 hours.

The mean arterial pressure (MAP) as a result of each of these treatments is summarized in Table 1.

Table 1

Effects of various ratios of [$(MGD)_2$/Fe] treatment on the mean arterial pressure (MAP in mmHg) in lipopolysaccharide (LPS)-induced shocked rats.

Conditions[1]	Baseline[2] (mean±SEM)	2 hrs after LPS Treatment	1.5 hrs after Treatment
a) Control saline (n=16)[3]	96±2	77±2	78±4
b) [$(MGD)_2$/Fe] (2/0.4)[4] (n=16)	95±3	75±2	96±3
c) [$(MGD)_2$/Fe] (2/0.2) (n=6)	98±3	73±4	87±4
d) MGD (2/0) (n=6)	102±5	73±2	94±6

1 Experimental conditions were as described in the text.
2 The values of MAP prior to LPS treatment.
3 n, the number of animals in each group.
4 [$(MGD)_2$/Fe] (2/0.4) is defined as the ratio of [$(MGD)_2$/Fe] to be 2-to-0.4.

The MAP of anesthetized rats was in the range of 96 to 102 mmHg. Two hours after LPS treatment, the MAP decreased to between 73 and 77 mmHg, which is indicative of the onset of systemic hypotension, caused by abnormally elevated levels of nitric oxide. While the 1.5 hr saline infusion did not change the MAP, infusions of [$(MGD)_2$/Fe] complex at various ratios, ranging from 2-to-0.4 (MGD to Fe) to 2-to-0 (MGD to Fe), restored the blood pressure to 87-96 mmHg (Table 1). These results suggest that the i.v. infusion of MGD either with or without added iron (Fe) can restore blood pressure in hypotensive rats induced by LPS challenge (Table 1).

Since MGD contains no reduced iron, it therefore cannot bind nitric oxide. The restoration of the MAP by the infusion of MGD can be attributed to the chelation by

MGD of the cellular iron released by excessive NO production, which is known to attack cellular iron-containing proteins and result in cellular iron loss during sepsis or septic shock (see, for example, Kim et al., in J. Biol. Chem. 270:5710-5713 (1995)). In other words, upon intravenous infusion, the MGD molecule binds to endogenous adventitious iron released from ferritin, transferrin or other iron-containing proteins to form the $[(MDG)_2/Fe]$ complex, which traps NO *in vivo*, forming the $[(MGD)_2/Fe\text{-}NO]$ complex. The heme group of hemoglobin in the red blood cell is not accessible to MGD inasmuch as the incubation of MGD with hemoglobin solution did not produce any measurable formation of the $[(MGD)_2/Fe]$ complex, presumably because of the high affinity of iron bound to the hemoglobin molecule. Thus, removal of free iron *in vivo*, which is known to cause oxidative damage to tissues, may be an additional advantage of the use of MGD as a therapeutic agent for the treatment of iron-overload or non iron-overload diseases and conditions, such as septic shock, hemodialysis, stroke, and thalassemia.

Example 5

Comparison of MGD and ICRF-187 for the promotion of hydroxy radical formation via the Fention reaction

The treatment of ICRF-187 was effective in removing iron from the anthracycline-iron complex, preventing the cardiac toxicity in patients with advanced breast cancer receiving 5-fluorouracil, doxorubicin and cyclophosamide (see, for example, Kolaric et al., *supra*). Once it has entered the cell, ICRF-187 is converted into ICRF-198, which chelates iron intracellularly to form Fe (ICRF-198) complex. The Fe (ICRF-198) complex was 150% as effective as noncyclic iron in promoting ·OH radical production in the Fenton reaction (see, for example, Thomas et al., *supra*). Using the same Fenton reaction as a chemical model system for hydroxyl radical production and

DMPO (i.e., 5,5-dimethyl-1-pyrroline N-oxide) as a spin trap reagent, it was found that ferrous iron chelated by MGD as the $[(MGD)_2/Fe]$ complex substantially reduces the reactivity of the iron species toward hydrogen peroxide to generate ·OH radical, compared to free ferrous iron. These results suggest that the $[(MGD)_2/Fe]$ complex is less likely to cause oxidative damage in tissues compared to the Fe (ICRF-198) complex.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

That which is claimed is:

1. A method for the *in vivo* reduction of free iron ion levels in a subject, said method comprising:

administering to said subject an effective amount of at least one physiologically compatible compound capable of binding free iron ions.

2. A method according to claim 1 wherein said physiologically compatible compound is a dithiocarbamate.

3. A method according to claim 2 wherein said dithiocarbamate has the formula:

$$[R_1R_2N\text{-}C(S)\text{-}S^-]\ M^{+1\text{ or }+2} \qquad (I)$$

wherein:

each of R_1 and R_2 is independently selected from a C_1 up to C_{18} alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocyclic, substituted heterocyclic, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, aroyl, substituted aroyl, acyl or substituted acyl, or

R_1 and R_2 can cooperate to form a 5-, 6- or 7-membered ring including N, R_1 and R_2, or

R_1 or R_2 is a divalent moiety selected from the group consisting of alkylene, substituted alkylene, oxyalkylene, substituted oxyalkylene, alkenylene, substituted alkenylene, arylene, substituted arylene, alkarylene, substituted alkarylene, aralkylene, substituted aralkylene,

aralkenylene, substituted aralkenylene, aralkynylene, substituted aralkynylene, cycloalkylene, substituted cycloalkylene, heterocycloalkylene or substituted heterocycloalkylene, wherein said divalent moiety serves as the same substituent for two dithiocarbamate structures, thereby linking said structures together so as to form a bis(dithiocarbamate) species, and

M is a monovalent or divalent cation.

4. A method according to claim 3 wherein:

each of R_1 and R_2 = a C_1 up to C_{12} alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl, wherein the substituents are selected from carboxyl, -C(O)H, oxyacyl, phenol, phenoxy, pyridinyl, pyrrolidinyl, amino, amido, hydroxy, nitro or sulfuryl, or

R_1 and R_2 can cooperate to form a 5-, 6- or 7-membered ring including N, R_1 and R_2, or

R_1 or R_2 is a divalent moiety selected from the group consisting of alkylene, substituted alkylene, oxyalkylene, substituted oxyalkylene, alkenylene, substituted alkenylene, arylene, substituted arylene, alkarylene, substituted alkarylene, aralkylene, substituted aralkylene, aralkenylene, substituted aralkenylene, aralkynylene, substituted aralkynylene, cycloalkylene, substituted cycloalkylene, heterocycloalkylene or substituted heterocycloalkylene, wherein said divalent moiety serves as the same substituent for two dithiocarbamate structures, thereby linking said structures together so as to form a bis(dithiocarbamate) species, and

$M = H^+$, Na^+, Zn^{+2}, Ca^{+2} or Mg^{+2}.

5. A method according to claim 3 wherein:

R_1 = a C_2 up to C_{10} alkyl or substituted alkyl, wherein the substituents are selected from carboxyl, acetyl, pyridinyl, pyrrolidinyl, amino, amido, hydroxy or nitro,

R_2 is selected from a C_1 up to C_6 alkyl or substituted alkyl, or

R_2 can cooperate with R_1 to form a 5-, 6- or 7-membered ring including N, R_2 and R_1, or

R_1 or R_2 is a divalent moiety selected from the group consisting of alkylene, substituted alkylene, oxyalkylene, substituted oxyalkylene, alkenylene, substituted alkenylene, arylene, substituted arylene, alkarylene, substituted alkarylene, aralkylene, substituted aralkylene, aralkenylene, substituted aralkenylene, aralkynylene, substituted aralkynylene, cycloalkylene, substituted cycloalkylene, heterocycloalkylene or substituted heterocycloalkylene, wherein said divalent moiety serves as the same substituent for two dithiocarbamate structures, thereby linking said structures together so as to form a bis(dithiocarbamate) species, and

$M = H^+$, Na^+, Zn^{+2}, Ca^{+2} or Mg^{+2}.

6. A method according to claim 3 wherein

R_1 = a C_2 up to C_8 alkyl or substituted alkyl, wherein the substituents are selected from carboxyl, acetyl, amido or hydroxy,

R_2 = a C_1 up to C_4 alkyl or substituted alkyl, or

R_1 or R_2 is a divalent moiety selected from the group consisting of alkylene, substituted alkylene, oxyalkylene, substituted

oxyalkylene, cycloalkylene, substituted cycloalkylene, heterocycloalkylene or substituted heterocycloalkylene having in the range of about 4 up to 11 carbon atoms, wherein said divalent moiety serves as the same substituent for two dithiocarbamate structures, thereby linking said structures together so as to form a bis(dithiocarbamate) species, and

$M = H^{+}$, Na^{+}, Zn^{+2}, Ca^{+2} or Mg^{+2}.

7. A method according to claim 1 wherein said elevated level of free iron ions is associated with a hereditary condition or an affliction related to invasive exchange of body fluids.

8. A method according to claim 1 wherein said elevated level of free iron ions is associated with anthracycline anti-cancer therapy, inflammation, septic shock, hemorrhagic shock, anaphylactic shock, toxic shock syndrome, arthritis, ulcers, ulcerative colitis, inflammatory bowel disease, gastritis, adult respiratory distress syndrome, asthma, cachexia, transplant rejection, myocarditis, multiple sclerosis, diabetes mellitus, autoimmune disorders, eczema, psoriasis, glomerulonephritis, heart failure, heart disease, atherosclerosis, Crohn's disease, dermatitis, urticaria, ischemia, cerebral ischemia, systemic lupus erythematosis, AIDS, AIDS dementia, chronic neurodegenerative disease, chronic pain, priapism, cystic fibrosis, amyotrophic lateral sclerosis, schizophrenia, depression, premenstrual syndrome, anxiety, addiction, migraine, Parkinson's disease, Huntington's disease, epilepsy, neurodegenerative disorders, gastrointestinal motility disorders, obesity, hyperphagia, ischemia/reperfusion injury, allograft rejection, solid tumors, malaria, cancers, Alzheimer's disease, infection, myelofibrosis, lung injury, graft-

versus-host disease, head injury, CNS trauma, cirrhosis, hepatitis, renal failure, liver disease, drug-induced lung injury, transplant rejection and preservation, burn, administration of cytokines, overexpression of cytokines, encephalomyelitis, meningitis, pancreatitis, peritonitis, vasculitis, lymphocytic choriomeningitis, uveitis, ileitis, myasthenia gravis (MG), ophthalmic diseases, post-angioplasty, restenosis, angina, coronary artery disease, stroke, chronic fatigue syndrome, photoaging or photodamage.

9. A method according to claim 1 wherein said elevated level of free iron ions is associated with anthracyline anti-cancer therapy.

10. A method according to claim 1 wherein said elevated level of free iron ions is associated with inflammation.

11. A method according to claim 1 wherein said elevated level of free iron ions is associated with infection.

12. A method according to claim 1 wherein said elevated level of free iron ions is associated with cytokine therapy.

13. A method according to claim 1 wherein said elevated level of free iron ions is associated with photoaging and/or photodamage.

14. A method according to claim 1 wherein said iron ion scavenger is administered in combination with an anti-inflammatory agent, an immunosuppressant, an antistroke agent, an anticancer agent, a thrombolytic agent, a neuroprotectant, a nitric oxide synthase inhibitor, an anti-migraine agent, a cytokine, an

antibiotic, a vasoactive agent, or mixtures of any two or more thereof.

15. A method according to claim 14 wherein said iron ion scavenger is administered in combination with a cytokine, an antibiotic, a vasoactive agent, or mixtures thereof.

16. A method according to claim 15 wherein said cytokine is selected from interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-12 (IL-12), tumor necrosis factor (TNF) or interferon-gamma (IF-γ).

17. A method according to claim 15 wherein said vasoactive agent is selected from a catecholamine, noradrenaline, dopamine or dobutamine.

18. A method according to claim 1 wherein said iron ion scavenger is delivered orally, topically, intravenously, subcutaneously, parenterally, rectally or by inhalation.

19. A method according to claim 18 wherein said topically applied iron ion scavenger is incorporated into a cosmetic formulation.

20. A method according to claim 1 wherein said iron ion scavenger is delivered in the form of a solid, solution, emulsion, dispersion, micelle or liposome.

21. A method for treating a subject having elevated circulating levels of free iron ions, said method comprising:

administering to said subject an effective amount of at least one physiologically compatible compound capable of binding free iron ions.

22. A method for treating overproduction of free iron ions in a subject, said method comprising:

administering to said subject an effective amount of at least one physiologically compatible compound capable of binding free iron ions.

23. A composition comprising adriamycin or liposomal adriamycin, plus dithiocarbamate.

Figure 1A

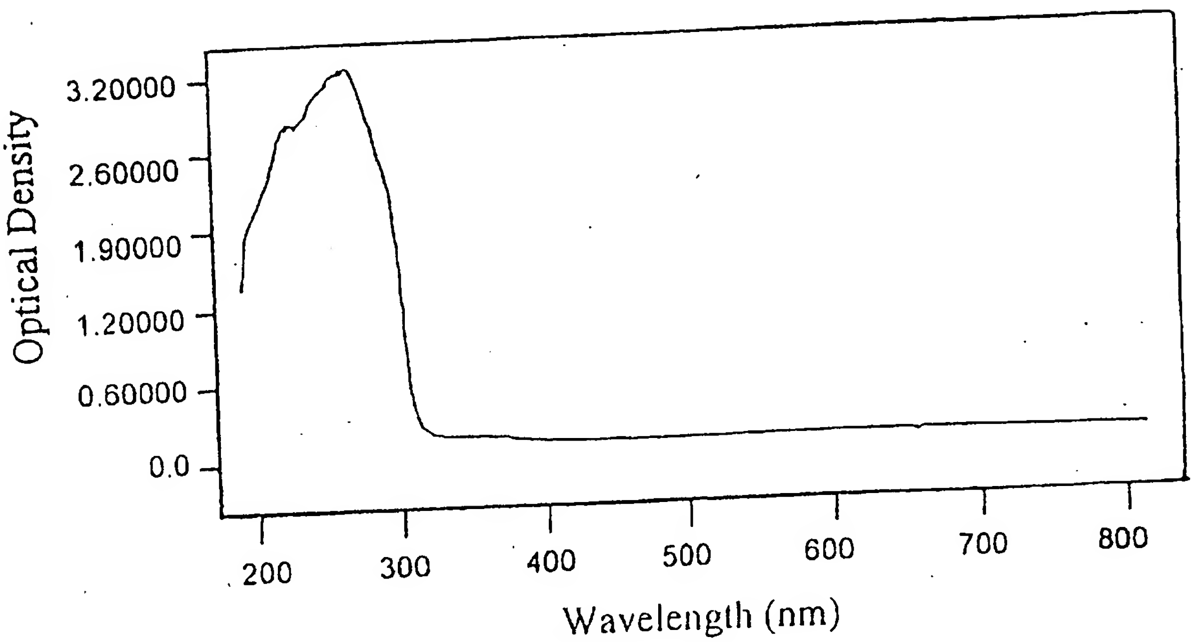


Figure 1B

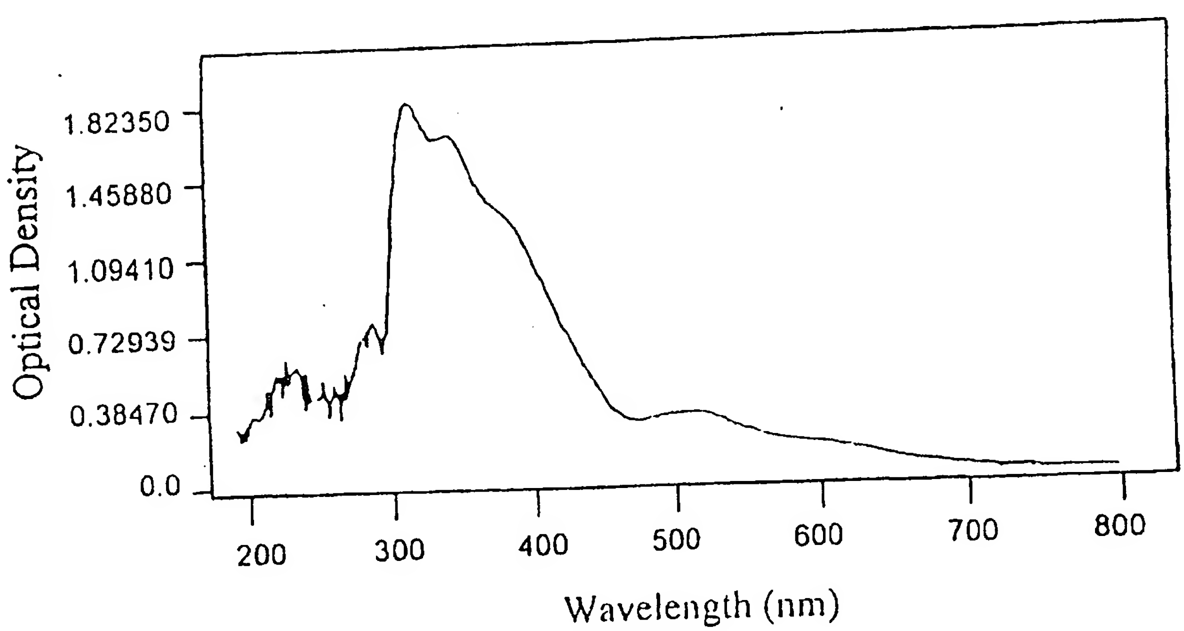


FIGURE 2

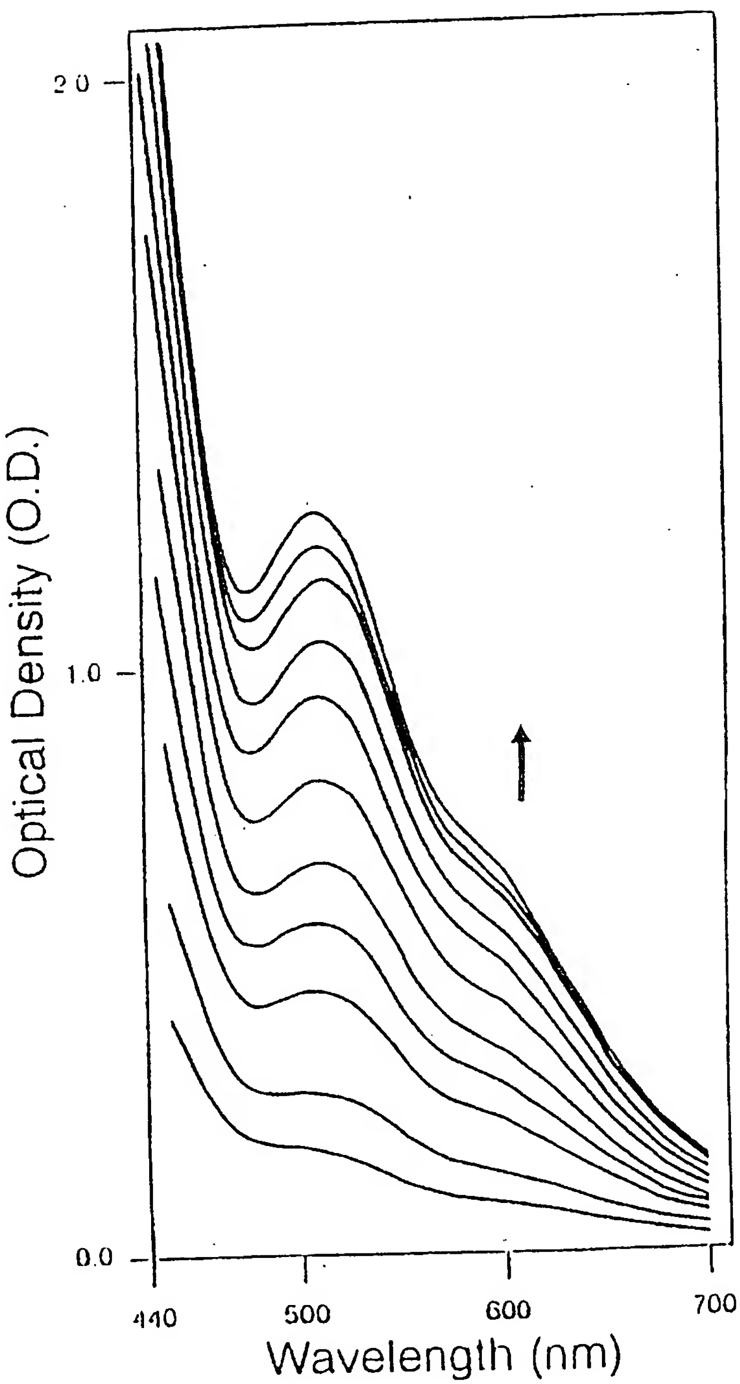
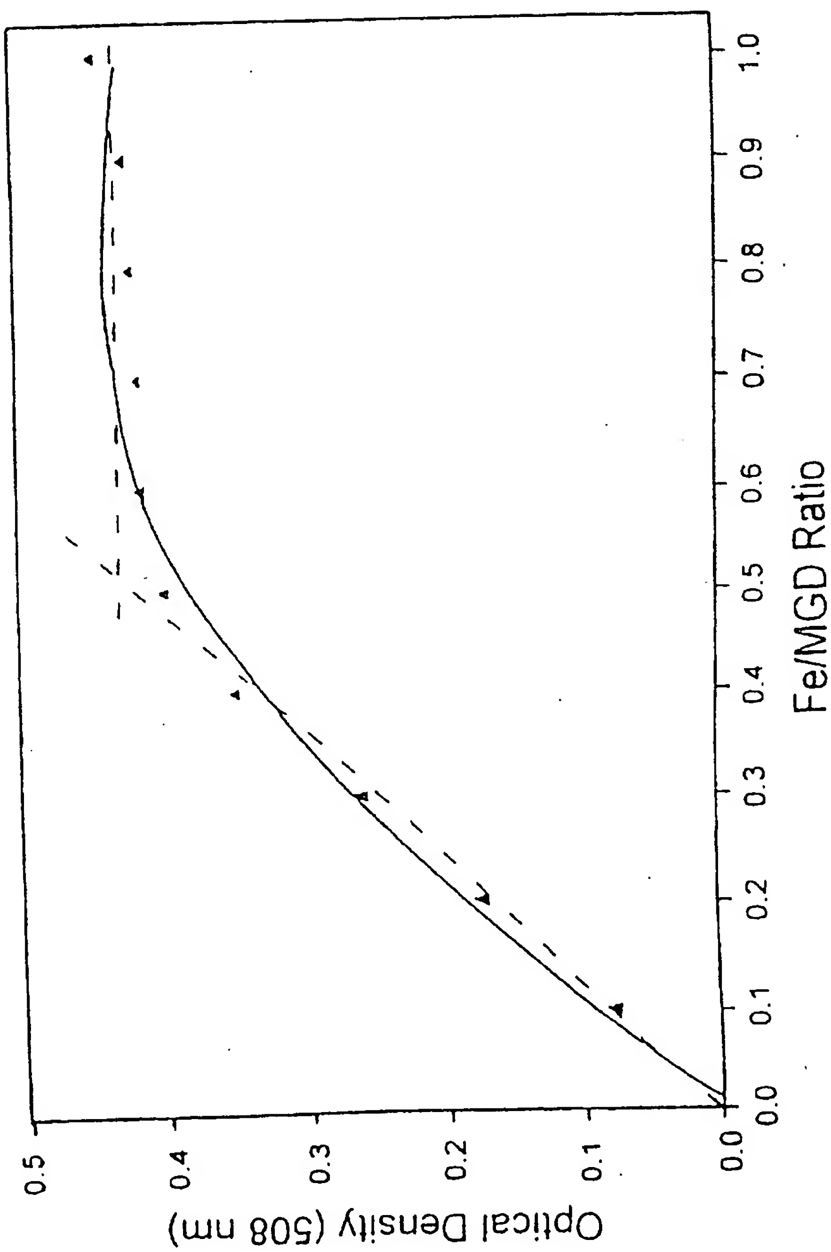


Figure 3


Optical Density (508 nm)
0.0
0.1
0.2
0.3
0.4
0.5
Fe/MGD Ratio
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/15325

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :514/423
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/423

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: dithiocarbamate, anemia, thalassemia, blood, cancer, Parkinson.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,056,621 A (BROWN et al.) 01 November 1977, column 1, line 12 to column 4, line 38.	1-23
Y	US 4,173,644 A (BROWN et al.) 06 November 1979, column 1, line 9 to column 4, line 46.	1-23
Y	US 4,894,393 A (NGUYEN et al.) 16 January 1990, column 1 line 9 to column 3, line 12.	1-23
Y	US 5,430, 058 A (SHANZER et al.) 04 July 1995, column 1, line 11 to column 2, line 20.	1-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

03 NOVEMBER 1997

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

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PCT/US97/15325

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/40